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Nicotine Metabolic Profile in Man: Comparison of Cigarette Smoking and Transdermal Nicotine[1]

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ABSTRACT

The objectives of this study were to 1) quantitatively assess human exposure to various metabolites of nicotine, 2) examine the influence of inhalation *vs.* transdermal administration on the patterns of nicotine metabolism, and 3) assess the extent of recovery of nicotine as various metabolites in people whose systemic intake of nicotine has been measured. Twelve smokers were studied while smoking cigarettes and while receiving transdermal nicotine. Urinary excretion of nicotine and eight of its metabolites was measured under steady state conditions. The systemic intake of nicotine in these subjects was determined using plasma concentrations and intravenous clearance data, so the percentage of their daily dose of nicotine excreted as various metabolites could be computed. The major findings of the study are as follows: 1) a high percentage (averaging [illegible]) of a systemic dose of nicotine can be accounted for by measurement of nicotine and its metabolites; 2) the pattern of metabolism is generally similar when nicotine is inhaled or absorbed transdermally; 3) while there is considerable interindividual variability in the pattern of metabolism, the pattern is consistent for an individual; and 4) within individuals, the extent of conjugation of nicotine and cotinine is highly correlated, but neither is correlated with the extent of conjugation of 3'-hydroxycotinine. This suggests that similar enzymes are involved in the conjugation of nicotine and cotinine, and that a different enzyme may be involved in the conjugation of 3'-hydroxycotinine.

Nicotine is the addictive principle of tobacco and may contribute to some of the injurious effects of tobacco use (Benowitz, 1988). Nicotine is also marketed as a pharmaceutical agent for use in the treatment of tobacco addiction and is being evaluated for treatment of other medical diseases.

Nicotine is extensively metabolized, primarily in the liver but also in the lung, resulting in a variety of metabolites (fig. 1) (Jacob and Benowitz, 1991; Turner *et al.*, 1975). Some of these metabolites appear to be pharmacologically active; for most, however, such activity has not been assessed (Clark *et al.*, 1965).

The pattern of metabolism of a drug may be influenced by the route of administration. For example, oral estradiol results in the generation of different levels of metabolites compared to those observed after transdermal dosing (Powers *et al.*, 1985). This difference in metabolism may be of clinical importance because the hepatic metabolites of estradiol may have adverse effects on lipid metabolism and, therefore, on cardiovascular risk. Pulmonary metabolism could represent a first-pass clearance mechanism for nicotine following tobacco smoking, and, conceivably, metabolic patterns could differ between inhaled and transdermal nicotine delivery.

The measurement of urine levels of cotinine, a major metabolite of nicotine, has been used widely as a biomarker of nicotine intake and, therefore, of tobacco exposure (Benowitz, 1984). However, cotinine in the urine accounts for less than 15% of the total systemic dose of nicotine (Benowitz *et al.*, 1983). A more complete quantitative recovery of nicotine metabolites in the urine could enhance the accuracy of urine assays as indicators of systemic nicotine exposure.

The objectives of the present study were: 1) to quantitatively assess human exposure to various metabolites after intake of nicotine, 2) to examine the influence of inhalation *vs.* transcutaneous dosing on the patterns of nicotine metabolism, and 3) to assess the extent of recovery of nicotine as various metabolites in people whose systemic intake of nicotine has been measured. Specifically, we have measured urinary excretion of nicotine and eight of its metabolites under steady-state conditions in people smoking cigarettes and in the same individuals receiving transdermal nicotine. The systemic intake of nicotine in these subjects was determined using plasma concentrations and intravenous clearance data, so the percentage of their daily dose of nicotine excreted as various metabolites could be computed.

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ABBREVIATIONS: HPLC, high performance liquid chromatography; GC-MS, gas chromatography-mass spectrometry; AUC, area under the plasma concentration time curve.

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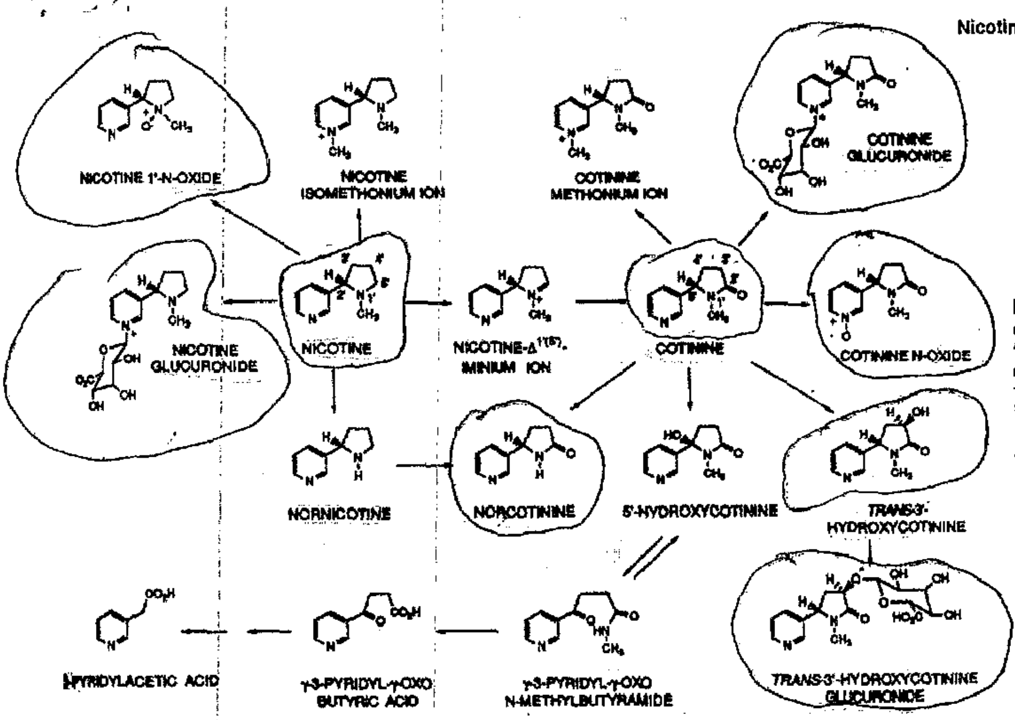

Fig. 1. Metabolic scheme, including structures of nicotine and the various metabolites discussed in this paper. The structures of nicotine and *trans*-3'-hydroxycotinine glucuronide are probable but not yet definitively established (see Discussion).

Methods

Subjects. Thirteen healthy adult male smokers who had smoked cigarettes for at least 1 year participated in the study. In one subject, urine collection was unsuccessful, so the results on 12 subjects' data are presented. The subjects ranged in age from 20 to 55 (mean age, 34 years), typically smoking 17 to 70 cigarettes per day (mean, 31 per day), with a history of smoking for 1 to 40 years (mean, 17 years). Subjects were confirmed to be in good health by physical examination, medical history, and routine clinical tests. Written consent was obtained from each subject. The study was approved by the Committee on Human Research at the University of California, San Francisco.

Experimental protocol. Subjects were confined in the Drug Studies Unit at the University of California, San Francisco, for 13 days. On days 1 and 2, subjects smoked their chosen brand of cigarettes *ad libitum*. From 08:00 on day 3, and for the remainder of the study, no smoking was permitted. Compliance with nonsmoking was assessed by periodic measurements of expired breath carbon monoxide concentrations. On day 5, beginning at 08:00, a 24-hr infusion of deuterium-labeled nicotine ((*S*)-3',3'-dideuteronicotine as the bitartrate salt) at a rate of 0.2 μg/kg/min was initiated. At the same time, a 22-cm² nicotine transdermal system (Nicoderm, Alza Corporation and Marion Merrell Dow, Inc.) was applied to the skin as part of a bioavailability study (the results of which are reported elsewhere (Gupta *et al.*, 1993)). This system includes a reservoir with nicotine in a copolymer matrix which is in contact with a rate-controlling polyethylene membrane. For five additional days (from day 7 through day 11), nicotine transdermal systems were applied to the skin daily. The patches were applied to rotating sites, including the upper chest, upper back and upper and outer arms.

Blood samples were taken at frequent intervals and urine was collected over 24 hr on days 2 and 11, during *ad libitum* cigarette smoking and the fifth day of transdermal nicotine, respectively. For measurement of clearance, blood samples for measurement of nicotine-d_2 concentrations were obtained at frequent intervals during and for 12 hr after intravenous infusion of nicotine-d_2, administered on day 5, as noted previously.

Analytical chemistry. Cotinine N-oxide concentrations in urine were measured by high performance liquid chromatography (HPLC) (Shulgin *et al.*, 1987). Nicotine and all other metabolite concentrations were determined by gas chromatography-mass spectrometry (GC-MS). Nicotine, nicotine-3'-3'-d_2, cotinine and *trans*-3'-hydroxycotinine were determined by published methods (Jacob *et al.*, 1991, 1993). Concentrations of nicotine 1'-N-oxide were measured by a modification of a GC method (Jacob *et al.*, 1986) in which nicotine 1'-N-oxide-d_3 is used as an internal standard. This assay measures total nicotine 1'-N-oxide and does not distinguish the *cis* and *trans* isomers. A recent study indicates that nicotine 1'-N-oxide excreted in humans is largely, if not entirely, the *trans*-nicotine 1'-N-oxide (Park *et al.*, 1993). Nornicotine was determined by a modification of a GC method (Zhang *et al.*, 1990) using nornicotine-d_4 as an internal standard.

Glucuronide-conjugated nicotine, cotinine and *trans*-3'-hydroxycotinine were measured as the differences in total concentrations before and after alkaline hydrolysis (nicotine and cotinine) or hydrolysis by incubation with β-glucuronidase (3'-hydroxycotinine). Base hydrolysis was performed by adding 0.4 ml of 4.0 M sodium hydroxide to a 1.0-ml urine sample to which the internal standards nicotine-d_4 and cotinine-d_4 had been previously added. The samples were placed onto a dry heating block at 75°C for 35 min. Nicotine and cotinine concentrations were then measured by GC-MS, as noted above. Control experiments showed that no further release of nicotine and cotinine occurred with more prolonged heating or at higher temperature, and that complete conversion of synthetic cotinine glucuronide (supplied by Dr. Peter Crooks; Caldwell *et al.*, 1992) to cotinine occurred under these conditions. Enzymatic hydrolysis was performed using a slight modification of the method described by Curvall *et al.* (1991). To 0.5-ml urine samples diluted with 0.5 ml of 0.05 M acetic acid buffer, pH 4.8, 6000 U of β-glucuronidase (EC 3.2.1.31, from *Helix pomita*, Sigma Chemical Co., St. Louis, MO) in 0.5 ml of acetic acid buffer at pH 4.0 were added and the samples were placed onto a dry heating block at 37°C for 24 hr. *trans*-3'-Hydroxycotinine concentrations were then measured by GC-MS. In control experiments, there was an excellent correlation between base and enzymatic deconjugation of nicotine and cotinine glucuronides.

Data analysis. Clearance of nicotine-d_2 was calculated as dose/area under the plasma nicotine-d_2 concentration time curve ($AUC_{nic}.d_2$). $AUC_{nic}.d_2$ was computed by the trapezoidal rule. The terminal area of $AUC_{nic}.d_2$ was calculated as the last nicotine-d_2 concentration/k, where k is the terminal slope of the nicotine-d_2 log/concentration time curve

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The 24-hr dose of nicotine (D) systemically absorbed by cigarette smoking or transdermal nicotine was determined using the AUC for natural nicotine (AUC_{nic}) and the average (24 hr) clearance of labeled nicotine ($CL_{nic}.d_2$) according to the equation (Benowitz *et al.*, 1991):

$$D = AUC_{nic} \times CL_{nic}.d_2$$

AUC_{nic} was measured by the trapezoidal method over 24 hr. The estimation of dose assumed that the levels represented steady-state values. Smokers had been smoking *ad libitum* prior to and for the first 2 days of the study, so steady state was presumed in the smoking condition. In the transdermal nicotine condition, plasma and urine measurements were made after 5 days of treatment which, based on known half-lives, is an adequate time to achieve steady state for nicotine and all of its metabolites.

A detailed analysis of the bioavailability and absorption kinetics of transdermal nicotine from this study has been published elsewhere (Gupta *et al.*, 1993).

Urine metabolite data were analyzed in several ways. First, the data were expressed as excretion per gram of creatinine. The values were normalized for creatinine because in a few cases the 24-hr urine volumes and creatinine levels were obviously low, indicating incomplete urine collection. The urine metabolite data were also examined as the molar fraction of all recovered nicotine plus metabolites, as well as the molar fraction of the systemic dose of nicotine, the dose computed as described above. For the latter analysis, to correct for incomplete urine collections, excretion data for each metabolite were normalized to the greater of the 24-hr urine creatinine excretion values for each individual subject. The rationale for this correction was that undercollection was apparent for several subjects, while overcollection is unlikely. Comparison of values on smoking and transdermal nicotine treatment days were made by paired *t* or Wilcoxon tests, as appropriate.

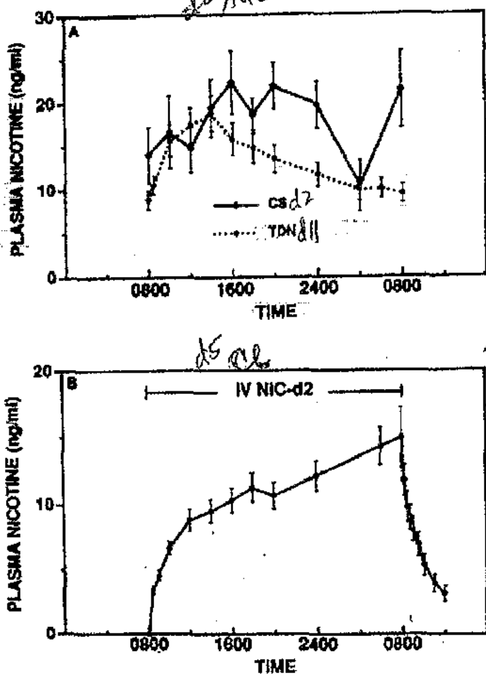

Fig. 2. A, mean plasma nicotine concentrations after 5 days of transdermal nicotine (TDN) dosing *vs.* cigarette smoking (CS). B, mean plasma nicotine-d_2 concentrations during and after intravenous nicotine-d_2 (IV NIC-d_2). Time indicates military clock time. Bars indicate S.E.M.

Results

Plasma concentrations of nicotine during cigarette smoking and transdermal nicotine are shown in figure 2A, and concentrations of labeled nicotine during intravenous infusion of nicotine-d_2 in figure 2B. Clearance values (derived from the infusion of nicotine-d_2 on day 5) and estimates of systemic absorption of nicotine from smoking and transdermal nicotine (on days 2 and 11 of this study, respectively) are given in table 1. For most subjects, the daily intake of nicotine while smoking was greater than the intake while wearing transdermal systems, mean 34.2 and 21.5 mg, respectively ($P < .05$, Wilcoxon test).

Metabolite excretion data for all subjects in the two treatment conditions are presented in table 2. Urine volumes averaged 1815 and 2179 ml/24 hr, and creatinine excretion 1767 and 1620 mg/24 hr, in the smoking and transdermal nicotine conditions, respectively. The difference in the mean values is not statistically significant. Most subjects had similar creatinine excretion in the two experimental conditions, but subjects 1, 4, 5 and 11 had substantially different values. For these subjects, there was evidence on the nursing records of incomplete urine collection on the days with lower creatinine values. The actual values for urine creatinine excretion on smoking and transdermal nicotine days are given in table 2.

The molar fractions of total nicotine plus metabolites recovered as individual metabolites in the two treatment conditions are summarized in table 3. For the major metabolites, the excretion pattern was similar in the two conditions. The fractional excretion of nornicotine was significantly greater and that of nicotine 1′-N-oxide tended to be greater during cigarette smoking, while the fractional excretion of cotinine N-oxide was significantly greater with transdermal nicotine treatment.

The excretion of various metabolites as a fraction of systemic dose of nicotine in the two treatments is shown in figure 3. During cigarette smoking, on average, 98% of the dose could be accounted for in the urine, with a range of 56 to 164% (table 1). In the transdermal nicotine condition, an average of 88% of the dose was recovered, with a range of 51 to 133%. Based on the transdermal nicotine treatment condition (results based on cigarette smoking condition were similar), the percentage of dose recovered as free plus conjugated 3′-hydroxycotinine averaged 41% (range 18–77), free plus conjugated cotinine 26% (range 17–36), free plus conjugated nicotine 14% (range 8–21). The combination of free plus conjugated cotinine and 3′-hydroxycotinine accounted for an average of 67% (range 37–104) of the dose.

41%
+ 26%
+ 14%
81%

Nicotine, cotinine and 3′-hydroxycotinine were 29.4, 51.0 and 16.4% conjugated, respectively (table 4). There was considerable individual variability in excretion patterns, including the fractions of nicotine, cotinine and 3′-hydroxycotinine that were conjugated, but there was consistency in the pattern for an individual comparing the two treatment conditions. As seen in figure 4, in some subjects (for example, A and B), total (free plus conjugated) 3′-hydroxycotinine is the predominant metabolite in the urine. In other subjects (C and D), total 3′-hydroxycotinine and cotinine excretion are similar to one another in magnitude. In subject F, total cotinine excretion exceeded that of 3′-hydroxycotinine. In subject A, there was virtually no conjugation of nicotine or cotinine, but 3′-hydroxycotinine was conjugated. In subject E, there was very little conjugation of 3′-hydroxycotinine, but nicotine and cotinine were conjugated. In subject F, cotinine was extensively conjugated, explaining why total cotinine excretion exceeded that of 3′-hydroxycotinine, while unconjugated excretion of 3′-hydroxycotinine exceeded that of unconjugated cotinine. Comparison of figure 4,

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TABLE 1
Nicotine clearance, systemic dose and urine recovery

Subject No.	Body Weight	Plasma Clearance	Systemic 24-h Dose of Nicotine (mg)		24-h Urine Recovery as Nicotine + Metabolites (% systemic dose)	
			Cigarette smoking	Transdermal nicotine	Cigarette smoking	Transdermal nicotine
	(kg)	(ml/min)				
1	74.0	2266	28.1	16.9	140	124
2	96.9	2158	46.0	12.8	144	100
3	65.8	961	20.7	16.8	164	133
4	84.9	786	23.1	23.8	115	85
5	74.4	1141	34.7	21.2	70	69
6	68.6	1223	25.7	24.7	61	89
7	76.1	1361	24.2	20.1	105	87
8	96.4	1210	19.8	22.0	99	80
9	67.6	1490	40.8	20.4	74	74
10	80.0	980	25.5	24.7	71	82
11	78.7	1541	97.4	25.2	76	82
12	73.8	1285	25.2	29.4	56	51
Mean	76.4	1367	34.2	21.5	98	88
S.D.	11.4	451	21.4	4.5	36	22

TABLE 2
Total urinary nicotine and metabolite excretion over 24 hr during *ad libitum* cigarette smoking (CS) and transdermal nicotine (TDN)*

Subject No.	Creatinine (g)		NIC (µg/g creat)		NIC-G (µg/g creat)		COT (µg/g creat)		COT-G (µg/g creat)	
	CS	TDN	CS	TDN	CS	TDN	CS	TDN	CS	TDN
1	2.73	1.93	1188	1132	47	0	2009	1309	28	0
2	2.58	2.11	1140	456	240	75	1549	641	2010	390
3	1.74	1.47	1539	1519	238	349	2809	2175	1667	1603
4	1.99	0.92	1441	1590	341	644	1805	1913	1518	1375
5	1.55	0.90	2213	1427	766	587	2064	2143	2447	1634
6	1.64	1.66	731	605	538	757	1495	1608	1844	2706
7	1.69	1.78	1116	596	437	376	1055	762	2166	1775
8	1.75	1.66	985	731	693	111	1145	1476	2145	2775
9	1.45	1.66	1428	642	626	342	3305	2107	2183	1057
10	1.33	1.55	749	1265	505	410	1727	1734	1834	1397
11	1.34	2.19	4265	1216	2325	841	2952	952	7218	2524
12	1.43	1.60	520	1015	662	569	1236	1363	2500	2064
Mean	1.77	1.62	1443	1017	618	505	1938	1516	2297	1609
S.D.	0.46	0.40	995	398	579	315	738	532	1678	859

*NIC, nicotine; NIC-G, nicotine glucuronide; COT, cotinine; COT-G, cotinine glucuronide; creat, creatinine.

Subject No.	3-HC (µg/g creat)		3-HC-G (µg/g creat)		NNO (µg/g creat)		CNO (µg/g creat)		NORNIC (µg/g creat)	
	CS	TDN	CS	TDN	CS	TDN	CS	TDN	CS	TDN
1	8375	4461	1603	1190	453	346	838	262	100	26
2	10900	2584	3109	1150	502	220	497	133	56	14
3	9334	6381	2888	1413	557	561	679	405	83	40
4	3410	2472	728	763	492	730	479	244	62	49
5	3458	2126	1446	380	919	642	539	173	66	22
6	5651	6942	321	696	544	521	436	350	61	46
7	7294	5575	55	281	420	436	482	347	56	21
8	2204	2962	693	813	599	801	341	225	63	46
9	9285	4143	979	1053	602	369	634	506	95	48
10	5438	5734	2834	1029	629	642	806	645	83	45
11	5881	2413	626	508	1446	407	934	60	162	21
12	3854	3843	190	519	591	663	360	370	48	39
Mean	6257	4136	1290	817	646	528	586	310	78	35
S.D.	2781	1687	1098	355	281	174	193	163	31	16

*3-HC, *trans*-3′-hydroxycotinine; 3-HC-G, *trans*-3′-hydroxycotinine glucuronide; NNO, nicotine 1′-N-oxide; CNO, cotinine *N*-oxide; NORNIC, nornicotine; creat, creatinine.

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TABLE 3

Average urinary excretion as molar percentage of total recovered nicotine and metabolites[a]

Metabolite	Cigarette Smoking	Transdermal Nicotine	95% C.I. of Difference
NIC	10.4 ± 4.4[b] 16%	11.1 ± 4.3	−1.6, 3.1
NIC-G	4.6 ± 2.9	5.3 ± 3.3	−0.2, 1.5
COT	13.3 ± 3.1 30	14.9 ± 4.6	−0.1, 3.3
COT-G	15.8 ± 7.8	15.4 ± 7.9	−1.9, 1.2
3-HC	39.1 ± 12.5 47	37.0 ± 10.8	−5.2, 1.0
3-HC-G	7.8 ± 5.9	7.9 ± 4.7	−3.0, 3.1
NNO	3.7 ± 0.9 8	2.7 ± 1.2	−0.01, 1.27
CNO	4.5 ± 1.5	5.2 ± 1.5	−1.8, −0.2
NORNIC	0.65 ± 0.15	0.41 ± 0.12	−0.3, −0.1

[a] C.I., confidence interval; NIC, nicotine; NIC-G, nicotine glucuronide; COT, cotinine; COT-G, cotinine glucuronide; 3-HC, *trans*-3′-hydroxycotinine; 3-HC-G, *trans*-3′-hydroxycotinine glucuronide; NNO, nicotine 1′-N-oxide; CNO, cotinine N-oxide; NORNIC, nornicotine.

[b] ± S.D.

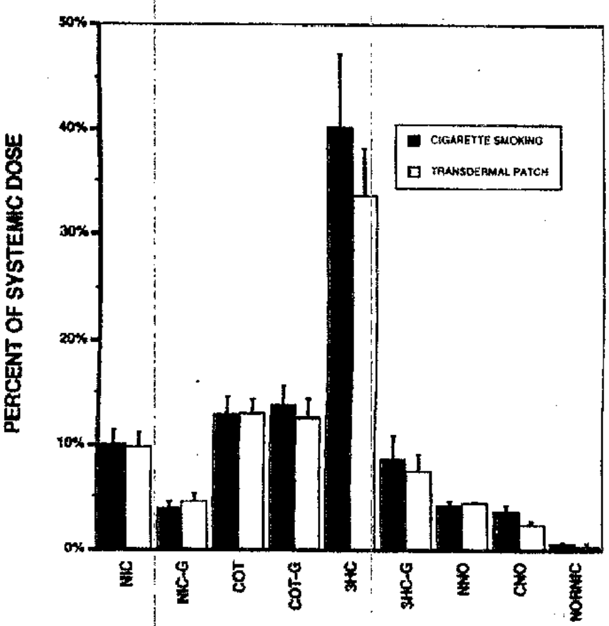

Fig. 3. Metabolite excretion as percent of systemic dose—comparison of cigarette smoking and transdermal nicotine. NIC, nicotine; NIC-G, nicotine glucuronide; COT, cotinine; COT-G, cotinine glucuronide; 3HC, *trans*-3′-hydroxycotinine; 3HC-G, *trans*-3′-hydroxycotinine glucuronide; NNO, nicotine 1′-N-oxide; CNO, cotinine N-oxide; NORNIC, nornicotine.

A and B, shows that the pattern of metabolism tended to be consistent for an individual in the cigarette smoking and transdermal nicotine conditions.

Supporting the consistency of pattern of metabolism for an individual in different treatments, a correlation analysis comparing the extent of conjugation and ratio of metabolites to precursors for individuals on smoking compared with transdermal nicotine days (using urine excretion data from table 2) revealed the following: percent of nicotine conjugation, $r = 0.74$ ($P < .01$); percent cotinine conjugation, $r = 0.93$ ($P < .01$); percent 3′-hydroxycotinine conjugation, $r = 0.48$ (not significant); ratio of total (conjugated plus unconjugated) cotinine/total nicotine, $r = 0.98$ ($P < .01$); and ratio of total 3′-hydroxycotinine/total cotinine, $r = 0.48$ (not significant).

To examine possible commonalities in mechanism of conjugation of different metabolites, a correlation analysis comparing percent conjugation of nicotine, cotinine and 3′-hydroxycotinine in individuals within treatment conditions was performed (table 5). In both conditions, the extent of conjugation of nicotine and cotinine was highly correlated, while neither nicotine nor cotinine conjugation was correlated with the extent of conjugation of 3′-hydroxycotinine.

Discussion

The major findings of this study are as follows: 1) a high percentage of a [illegible] cotine [illegible] unted for by measurement [illegible] tine and eight of it [illegible] tes; 2) the pattern of meta[illegible] enerally simil[illegible] nicotine is absorbed following [illegible] of tobacco s[illegible] or [illegible] absorbed transder[illegible] there is consider[illegible] individual variability [illegible] pattern of metabolism, [illegible] is consistent [illegible] dual when measured at different times; and 4) within [illegible] viduals, the extent of conjugation of nicotine and cotinine is highly correlated, but neither is correlated with the extent of conjugation of 3′-hydroxycotinine. This suggests that similar enzymes are involved in the conjugation of nicotine and cotinine, and that a different enzyme is involved in the conjugation of 3′-hydroxycotinine.

Several methodologic issues warrant discussion. We have assumed that our urine data reflect metabolite excretion under steady-state nicotine exposure conditions. Steady state almost certainly is achieved during transdermal nicotine treatment, where subjects were studied after 5 days of daily dosing. In the cigarette smoking condition, however, we have assumed that smokers smoked the same amount and consumed the same daily dose of nicotine when confined on the Drug Studies Unit as they had in the previous few days before entering the study. If subjects had smoked less on the day of the urine collection compared with the previous few days, the recovery of the metabolites with longer half-lives would be relatively greater than that expected based on the dose of nicotine on the urine collection day, consistent with the excretion of residual nicotine from previous day's exposure. Recovery of [illegible] bolites derived from a previous [illegible] s moking at a higher [illegible] se level migh[illegible] why the apparent [illegible] nicotine pl[illegible] [illegible]

In estimating the systemic dose of nicotine during cigarette smoking and transdermal nicotine, we have used the plasma nicotine concentrations taken on the day of urine collection and the intravenous clearance of deuterium-labeled nicotine measured on a different day. We assum[illegible] systemic [illegible] nicotine during [illegible] tine days is the [illegible] intra[illegible] infusion day. [illegible] of blood sampling, that is, every ½ to 4 hr o[illegible] likely to provide a good estimate of the area unde[illegible] concentration time curve for transdermal nico[illegible] levels of nicotine [illegible] and fall [illegible] This [illegible] AUC [illegible] where we are unable to [illegible] p[illegible] immediately [illegible]

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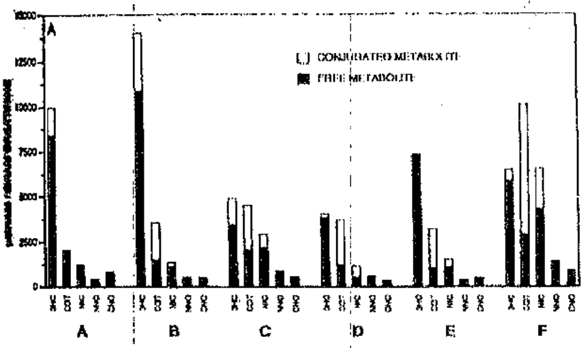
Conjugation of nicotine and metabolites during *ad libitum* cigarette smoking (CS) and transdermal nicotine (TDN)

	Nicotine		Cotinine		3-Hydroxycotinine	
	CS	TDN	CS	TDN	CS	TDN
% Conjugated[a]						
Mean	29.4	31.8	51.0	47.8	16.4	17.4
S.D.	14.6	16.8	19.0	20.2	10.6	6.9
Range	3.8–56.0	0–60.3	1.4–71.0	0–72.6	0.8–34.3	4.8–30.8
95% C.I.[b]	20.2–38.7	21.1–42.4	38.9–63.1	35.0–60.7	9.7–23.2	13.0–21.8
Ratio [conjugated / unconjugated]						
Mean	0.48	0.56	1.27	1.18	0.22	0.22
S.D.	0.34	0.44	0.71	0.81	0.16	0.10
Range	0.04–1.27	0–1.51	0.01–2.44	0–2.65	0.01–0.52	0.05–0.44
95% C.I.	0.26–0.69	0.28–0.84	0.82–1.72	0.67–1.70	0.11–0.32	0.15–0.28

[a] $\% \text{conjugated} = \dfrac{\text{conjugated}}{\text{conjugated + unconjugated}} \times 100.$

[b] C.I., confidence interval.



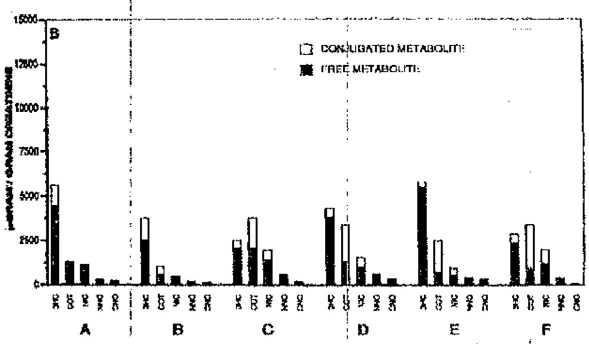


Fig. 4. A, metabolite excretion for six individuals—cigarette smoking. B, metabolite excretion for six individuals—transdermal nicotine. Letters identify individual subjects for comparison of cigarette smoking and transdermal nicotine conditions. See the legend to figure 3 for abbreviations.

smoking. Thus, we may have underestimated the systemic intake of nicotine during cigarette smoking, which could also explain why in some of our subjects the total recovery of nicotine metabolite exceeded 100% of the estimated systemic dose.

Our data indicate that *trans*-3′-hydroxycotinine is the major urinary metabolite of nicotine, accounting for an average of 38% of all metabolites. (Previous studies in our laboratory (Jacob *et al.*, 1990) and another laboratory (Voncken *et al.*, 1990) have indicated that only small amounts of *cis*-3′-hydroxycotinine are excreted in the urine of smokers.) Similar observations on the quantitative importance of 3′-hydroxycotinine have been made by several other investigators (Byrd *et al.*, 1992; Jacob *et al.*, 1991; Kyerematen *et al.*, 1990; Neurath *et al.*, 1987; Scherer *et al.*, 1988). Similar to previous studies (Benowitz *et al.*, 1983), we have also found that cotinine and nicotine account for 15 and 10% of urine metabolites, respectively. ?? 26 +14%

The conjugation of nicotine, cotinine and 3′-hydroxycotinine is another important route of nicotine metabolism, with conjugates accounting for another 25 to 30% of the total metabolites recovered. Hydrolysis by β-glucuronidase (Curvall *et al.*, 1991) has provided evidence that the conjugates of nicotine and *trans*-3′-hydroxycotinine are glucuronides. The conjugate of cotinine has recently been synthesized and identified as an N-glucuronide by Caldwell *et al.* (1992). The extent of conjugation of nicotine and metabolites has been previously studied by Curvall *et al.* (1991) and Byrd *et al.* (1992). Both groups used β-glucuronidase to deconjugate nicotine and its metabolites, which were then quantitated by GC or liquid chromatography-mass spectrometry. We used base hydrolysis to deconjugate nicotine and cotinine, and β-glucuronidase to deconjugate 3′-hydroxycotinine, and we used GC-MS for quantitation. Despite the different methods used, our data on various metabolites and total metabolite recovery were similar to those reported in the other studies. Specifically, our average ratios for conjugated to unconjugated nicotine, cotinine and 3′-hydroxycotinine averaged 0.5, 1.2 and 0.2, as compared to 0.25, 1.1 and 0.25 reported by Byrd *et al.* and 1.0, 2.0 and 0.4 reported by Curvall *et al.*

Our data and those of Byrd *et al.* (1992) show a great deal of interindividual variability in the percent excretion of various

TABLE 5

Correlation table comparing extent of conjugation of nicotine, cotinine and 3′-hydroxycotinine within individuals[a]

	Cigarette Smoking		Transdermal Nicotine	
	NIC-G[a]	COT-G	NIC-G	COT-G
	%		%	
% COT-G[b]	0.72*	—	0.82**	—
% 3-HC-G	−0.25	0.07	−0.42	−0.50

* P < .05.
** P < .01.

[a] $\% \text{ conjugated} = \left[\dfrac{\text{excretion of glucuronide}}{\text{excretion of unconjugated + glucuronide}}\right] \times 100.$

[b] NIC-G, nicotine glucuronide; COT-G, cotinine glucuronide; 3-HC-G, *trans*-3′-hydroxycotinine glucuronide.

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metabolites, and in the extent of conjugation of nicotine, cotinine and 3′-hydroxycotinine. Because we studied nicotine metabolism at two different times, we were able to examine the consistency of patterns of excretion over time. Despite the different routes of absorption of nicotine, the extent of conjugation of nicotine and cotinine and the ratio of total cotinine to total nicotine in the urine were consistent for individuals in the two treatment conditions. The relationship between specific patterns of metabolism and the rate of elimination of nicotine, the latter of which may influence cigarette smoking behavior, remains to be elucidated.

Our data suggest that similar conjugating enzymes are involved in the conjugation of nicotine and cotinine. Inspection of metabolite patterns for different individuals (fig. 3) suggests that people who extensively conjugate nicotine also extensively conjugate cotinine, but that the conjugation of 3′-hydroxycotinine is unrelated to that of nicotine and cotinine. These relationships were confirmed by correlation analysis for the 12 subjects (table 5) and were found to be true in both the smoking and transdermal nicotine conditions. In developing our assays, we also found that both nicotine and cotinine can be deconjugated with alkaline hydrolysis as well as with β-glucuronidase, but 3′-hydroxycotinine was deconjugated only by β-glucuronidase. It has been reported (Dahl-Puustinen and Bertilsson, 1987) that N-glucuronides of other drugs are readily cleaved by base, in contrast to most O-glucuronides. It appears, therefore, that both nicotine and cotinine form pyridine N-glucuronides, and that the same enzyme is involved in both.

As mentioned above, it has been established that cotinine glucuronide is conjugated through the pyridine nitrogen. We have carried out experiments (Jacob and Shulgin, unpublished data) which indicate that nicotine, at least in part, is conjugated through the pyridine nitrogen. Treatment of smokers' urine with sodium borohydride followed by hydrolysis produced tetra- and hexahydro derivatives of nicotine, which strongly suggests a pyridine quaternary glucuronide, since it is known that quaternary salts of pyridine derivatives are readily reduced under these conditions, whereas uncharged pyridine derivatives are not. (Known metabolites of nicotine were not reduced under these conditions.) A recent study (Schepers *et al.*, 1992) provided evidence that 3′-hydroxycotinine is conjugated through oxygen, which may involve other enzymes.

One of the reasons we conducted this study was to determine if the pattern of metabolism of nicotine was different comparing cigarette smoking and transdermal nicotine. The pattern of metabolism, as seen in the excretion of the major metabolites as a percentage of total recovered metabolites, was very similar in the two conditions. There were differences in excretion of the minor metabolites, nicotine 1′-N-oxide and cotinine N-oxide, in smoking *vs.* transdermal nicotine conditions. Nicotine [illegible]N-oxide [illegible] flavin-containing [illegible] (Cashman *et al.*, [illegible] his enzyme is present to [illegible] extent in the lung (Williams *et al.*, 1990) and conceivably c[illegible]d result in some first-pass pulmonary formation and greater formation of nicotine 1′-N-oxide in the smoking condition. It is unclear why cotinine N-oxide excretion is lower in the smoking *vs.* transdermal nicotine condition.

Excretion of various metabolites as a percent of systemic intake of nicotine could be estimated because we had plasma nicotine data during cigarette smoking and transdermal nicotine, as well as systemic clearance data for each subject. Considering the data from the transdermal nicotine day, where we have the greatest confidence in the estimate of systemic dose, the average recovery was 88%. Our assays did not measure norcotinine (demethylcotinine), as reported by Byrd *et al.* (1992), nor have we assayed for nicotine isomethonium ion, cotinine methonium ion, metabolites resulting from degradation of the pyrrolidine ring (fig. 1) or metabolites of nornicotine. Presumably, these various metabolites account for the remainder of the nicotine. Of note, however, were three subjects in whom we could only account for [illegible] dose. Possibly, these [illegible] different patterns of nicotine metabolism.

Considering cotinine and all of its metabolites (*i.e.*, cotinine glucuronide, 3′-hydroxycotinine, 3′-hydroxycotinine glucuronide and cotinine N-oxide), it appears that on average 70% of the dose of nicotine is metabolized to cotinine. This finding is similar to that which we have estimated in previous publications (Benowitz *et al.*, 1990), but somewhat lower than the 85 to 90% that we have found in recent unpublished studies with dual infusion of nicotine and cotinine. Possibly, there are other metabolites of cotinine that are as yet unmeasured.

Nornicotine is of interest as it is present as an alkaloid in tobacco and is pharmacologically active (Risner *et al.*, 1988). In our previous studies with intravenous infusion of deuterium-labeled nicotine, we have documented that nornicotine is also a metabolite of nicotine (Jacob *et al.*, 1991). The present study, comparing cigarette smoking and transdermal nicotine, allows us to estimate how much nornicotine derives from tobacco *per se* rather than from the metabolism of nicotine. The urinary recovery of nornicotine as a percent of total nicotine metabolites was [illegible] and 0.41% during trans[illegible] it is possible that smoking accelerates the metabolism of nicotine to nornicotine in addition to nornicotine being absorbed from tobacco smoke. In any case, it appears that the majority of nornicotine excreted by smokers is derived from the metabolism of nicotine, and that 40% or less comes from tobacco *per se.*

Using the data from the transdermal nicotine day, we have developed a schema (fig. 5) which describes the quantitative disposition of nicotine in man. Our recovery data have practical implications for the use of nicotine metabolites as biomarkers of nicotine exposure. To date, most researchers have used

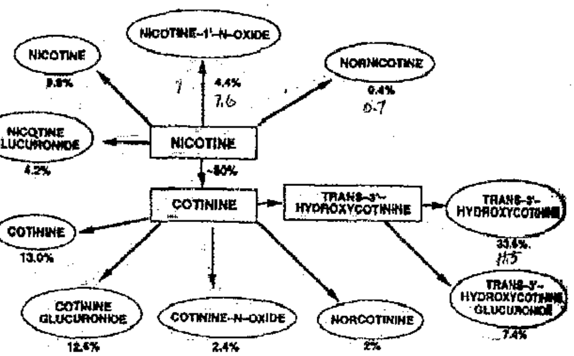

Fig. 5. Quantitative scheme of nicotine metabolism, based on average excretion of metabolites as percent of systemic dose during transdermal nicotine application. Circled compounds indicate excretion in urine and associated numbers indicate percent of systemic dose of nicotine. The estimate of percent conversion of nicotine to cotinine is based on unpublished studies with dual infusion of nicotine and cotinine. Estimates of norcotinine excretion are based on data of Byrd *et al.* (1992).

Source: https://www.industrydocuments.ucsf.edu/docs/yppj0001

cotinine levels (in plasma, urine or saliva) as a marker of the intake of nicotine (Benowitz, 1984). Our data indicate that there is much individual variability in urinary cotinine excretion as a percentage of the dose or as a percentage of total recovered metabolites. Some variability can occur owing to individual differences in percent conversion of nicotine (perhaps related to genetic or age differences in metabolic activity) or to differences in urine pH (which have a modest effect on cotinine excretion (Benowitz *et al.*, 1983)). However, our data indicate that a considerable source of variability derives from the fact that 50% of cotinine is conjugated, and that the degree of conjugation is variable from person to person. Also, the percent of cotinine converted to 3'-hydroxycotinine is variable from person to person. It is likely that measuring total, that is, conjugated plus unconjugated, cotinine would improve the accuracy of cotinine, and the measurement of total cotinine plus 3'-hydroxycotinine would improve even more the accuracy of metabolite concentration as a urine biomarker of systemic nicotine exposure.

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